RESEARCH PLAN PROPOSAL

Assessment of Nutritional Quality and Evaluation of Antioxidant and Antimicrobial Activities of *Rasbhari* (*Physalis peruviana*) and *Jungle jalebi* (*Pithecellobiumdulce)*

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Submitted by

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1. Introduction

1.1 Underutilized fruits

India is endowed with a rich genetic diversity of fruits. Fruits supplement human diet as they provide essential vitamins, minerals and fiber required for maintaining health. Certain therapeutic uses of fruits are also reported. Carotenoids and vitamin C are known for reducing the cancer risk by enhancing tumor surveillance by immune system (Macrae et al., 1993). Fruits contain high levels of biologically active components that impart health benefits beyond basic nutritional value. Within the biologically active components, natural antioxidants have attracted interest because of their safety and potential therapeutic effect (Rufino et al., 2011). The total production of fruits in World is reported as 2500 million metric tons (FAO, 2011).

India is a second largest producer of fruits after China, with a production of 68466 thousand metric tons of fruits from an area of 6101 thousand metric hectares (Mistry et al., 2010). According to National Horticulture Board (2010) the main fruits grown in India are apple, banana, lime, lemon, grapes, orange, mango and papaya. The fruits viz. mango, banana, guava, grape, pineapple and apple are the major ones. Apart from these, fruits like papaya, jackfruit, zizyphus and pomegranate fall under tropical and sub- tropical region whereas peach, pear, almond, walnut, apricot, strawberry and apples grow well in temperate region. Many of these fruits have got due attention since time immemorial and became popularized due to their larger appeal and ethos.

In addition to the major fruits, the various underutilized fruits are widely grown in India. Underutilized fruits are those which are neither cultivated in an organized farming system nor processed by established commercial processing methods. These fruits have many advantages in terms of easiness to grow, hardy in nature and production of good quality fruits even under adverse conditions. Most of these fruits are rich sources of vitamins, minerals and other nutrients. A large proportion of rural population depends on locally available fruits to meet their nutrient requirement. Most of these fruits have an exotic flavor and attractive color and have their own history of consumption. Local people are well aware about their nutritional and medicinal properties and the popularity of these fruits varies locality to locality which can be enhanced to a greater extent through publicity. The utilization of these fruits not only enhances

the components of one's food basket but also helps in increasing the beneficial effect of such fruits (Rai et al., 2008).

Consumption of underutilized fruits can provide nutrition to the poor and meet their nutrient requirements. These are a rich in carbohydrates, fats, proteins, energy, vitamin A, B_1, B_2, B_3, B_6, B_9, B_{12}, C, folic acid, minerals like Ca, P and Fe, dietary fiber and antioxidants. Thus they have the nutritional capacity to prevent and cure various diseases like kwashiorkor, marasmus, night blindness, anemia, diabetes, cancer, hypertension and hidden hunger. It is also established fact that seasonal, locally available and cheap fruits and vegetables can also keep the population healthy and nutritionally secure rather than costly off season ones (Nandlal & Bharadwaj, 2013). In India various types of underutilized fruits are available seasonally but are not utilized to the extent they should be, in spite of their higher nutritive value. Looking into the prevalence of high level of micronutrient malnutrition among the vulnerable sections of our society, consumption of less utilized fruits can be explored to overcome nutritional disorders (Joshi & Mathur, 2010)

1.1.1 **Underutilized fruits**

Rajasthan, situated in the northwestern part of India is the biggest state in the country. The physical features are characterized mainly by the Aravali mountain range and to some extent by the Vindhyan formation and the Deccan trap. A major portion of western Rajasthan has desert soils and sandy plains. Sand dunes occupy a greater part of western Rajasthan. The productivity of horticulture crops is largely influenced by geographical and environmental diversity prevailing in different zones of Rajasthan. The wide variation in altitude and other agro-climatic parameters such as rainfall and temperature, various fruits are grown in different zones of Rajasthan, which are being used by the local inhabitant. These fruits were an important source of food for mankind before dawn of civilization and domestication of present day fruit (Sharma, 2011).

Desert region is endowed with valuable natural resources with special mention of Arid fruits and vegetables. Arid foods are largely used by the native people of North West India as a prime source of food with their own traditional wisdom and with less effort for the value addition. However, processing of traditionally important arid foods into more useful and convenient product can improve lively hood security of the people residing in the desert regions (Goyal & Sharma, 2009).

The locally available fruits are cheap and highly nutritious and have been known for medicinal and therapeutic properties to cure various diseases. Not only the fruits, seeds but also leaves of the plant are used as curative foods in the traditional Indian medicine and Ayurveda. For example, Indian gooseberry (aonla) is used for treating diabetes, wood apple (*bae*l) fruit for beating the heat, *bael* leaf for diabetes, and *ber* and *phalsa* being highly rich in vitamin C are used in cases of vitamin C deficiency. Besides this, some of the fruits have an excellent flavour and taste and are used for preparing delicacies at home. eg. *ber, matira*, pickles made of *lasora*, or dishes made with *ker, sangri, kachri*, and *kumat* in Rajasthan (Rathore, 2009).

The interest of consumers towards lesser known wild edible fruits has arisen due to their various beneficial effects and organoleptic properties. These wild fruits have played an important role in supplementing the diet of rural people. Detailed information about the health promoting components of lesser known fruit species could lead to a better understanding of the beneficial effects and an increased consumption of these fruits, including their utilization as functional foods and as ingredients in nutraceuticals, medicines and pharmaceuticals (Yilmaz et al.,2009).

Singh et al., (2008) carried out a study to find out processing and marketing feasibility of underutilized fruit species of Rajasthan. The study indicated development of products such as squash, *chutney*, jam, ready -to-serve (RTS) fruit drinks and pickle using underutilized fruits like *ber, bael, jamun, karonda, phalsa, aonla*, mulberry, soursop and tamarind. The products were assessed for quality and marketing feasibility. The finding revealed the products to be well accepted. This indicated that there is significant potential for the production and marketing of underutilized fruits in Rajasthan.

1.2 Antioxidant properties

Antioxidants are special class of micronutrients (the term ‘micronutrient’ means that only miniscule amounts are required to provide essential support for vital metabolic functions). Antioxidants block harmful chemical reaction caused by oxidation, i.e. the destructive effect of oxygen and other oxidizing agents on the molecular components of cell. Antioxidants are substances which are both, nutrient *viz.* vitamin E, C, beta carotene; and non nutrient *viz.* plant phenols, flavonoids, etc. found naturally in many fruits and vegetables (Wiel & Low, 2014).

Oxygen centered free radicals and Reactive Oxygen Species (ROS) are highly toxic resulting in cell death and tissue damage leading to diseases such as atherosclerosis, diabetes, cancer, cirrhosis and neurodegenerative disorders. Antioxidants are compounds which can inhibit or delay the oxidative damage preventing many diseases (Shrikanta et al.,2013).

According to Saini et al., (2012) cell damage caused by free radicals appears to be a major contributor to aging and degenerative diseases such as cancer, cardiovascular diseases, cataracts, diabetes, immune system decline and brain dysfunction to protect the cell from damage caused by free radicals. A variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals

1.2.1 Various type of antioxidants

Vitamin C, vitamin E and beta carotene are among the most widely studied dietary antioxidants. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E, a major lipid-soluble antioxidant and is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin C has been cited as being capable of regenerating vitamin E. Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues.This research suggests that beta carotene works synergistically with vitamin E. A diet that is excessively low in fat may negatively affect beta carotene and vitamin E absorption, as well as other fat-soluble nutrients. Fruits and vegetables are major sources of vitamin C and carotenoids, while whole grains and high quality, properly extracted and protected vegetable oils are major sources of vitamin E (Percival, 1996).

1.2.2 Role of antioxidants

Antioxidants play an important role in overall health. They are natural compounds found in some foods that help neutralize free radicals in our bodies. Free radicals are substances that occur naturally in our bodies but attack the fats, protein and the DNA in our cells, which can cause different types of diseases and accelerate the aging process (IFT, 2013).

Antioxidants are the substances that reduce damage due to oxygen, such as that caused by free radicals. Well-known antioxidants include enzymes and other substances, such as vitamin C,

vitamin E and beta carotene, which are capable of counteracting the damaging effects of oxidation. Antioxidants are also commonly added to food products such as vegetable oils and prepared foods to prevent or delay their deterioration from the action of air. Antioxidants may possibly reduce the risks of cancer. Antioxidants clearly slow the progression of age-related macular degeneration (Jegtvig, 2014).

Antioxidant compounds present in food, play an important role by being health protecting factors. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Prakash, 2001).

Dadheech et al.,(2009) carried out a study on evaluation of antioxidant deficit in Schizophrenia, and the study concluded that an imbalance in the antioxidant defense system, along with enzymatic antioxidants, occurs in schizophrenia due to the persistent oxidative stress. Modern life style perhaps also contributes to the condition.

Shen et al., (2009) carried out a study on the protective effect of Zizyphus jujube fruit (FZJ) on carbon tetra chloride induced hepatic injury in mice by anti-oxidative activities. The study revealed that after administration of 200mg/kg dose FZJ, it significantly decreased alanine aminotransferase (ALT) and aspartate aminotransferase (AST), attenuated histopathology of hepatic injury and ameliorated the oxidative stress in hepatic tissue. Partly assayed indexes were ameliorated after administrated FZJ at the dose of 100mg/kg.

Underutilized fruits rich in phytochemicals including phenolic compounds exhibit antioxidant activity thereby offer opportunities for developing value-added products, such as functional drinks to enhance health benefits. The study conducted by Shrikanta et al., (2013) on antioxidant properties of underutilized fruits clearly suggested that Mulberry (*Morusrubra*), Indian blackberry (*Syzygiumcuminii*) and Jackfruit (*Artocarpusheterophyllus*) may be considered as sources of natural antioxidants and consumption of these fruits may supply substantial antioxidants which may provide health promoting and disease preventing effects. This study

suggests that underutilized fruits rich in antioxidants would help fight against heart related diseases and cancer and hence has tremendous scope for development of functional beverages.

1.2.3 Dietary sources of antioxidants

Synthetic antioxidants like butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are widely used but they have been restricted recently because of serious concerns about their side effects (Botterweck et al., 2000) Today there is interest in finding natural antioxidants without undesirable side effects and they should aid the endogenous protective system. The value of antioxidants in food has long been recognized for their health benefits. It is believed that higher intake of antioxidant rich fruits are associated with decreased risk of degenerative diseases particularly coronary heart disease (CHD) and cancer (Ruxton et al., 2006). The beneficial effect of fruits is mainly associated with the radical scavenging activity of polyphenols, which are largely present in fresh fruits and their processed products.

The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables and grains are rich sources of dietary antioxidants. Some dietary antioxidants are also available as dietary supplements (Bouayed & Bohn, 2010).

Table 1: Common fruit source of antioxidant per 100 gm (Rathore, 2009)

S. No.	Fruit	Vitamin A (IU)	Vitamin C(mg)
1	Apple	0	1
2	Banana	78	7
3	Orange	1104	30
4	Papaya	666	57
5	Grapes	0	1
6	Mango	2743	16
7	Dates (dried)	26	3

http://www.academicjournals.org/jhf/PDF/pdf2009/Sept/Rathore.pdf

Table 2: Underutilized fruit source of antioxidants per 100 gm (Rathore, 2009)

S.No.	Fruit	Vitamin A (IU)	Vitamin C (mg)

1	Bael (*Aeglemarmelos*)	91.6	1.1
2	Karonda (*Carissa spp.*)	1619	9-11
3	Aonla, Amla (*Emblicaofficinalis*)	17	500-625
4	Phalsa (*Grewiasubinaequalis*)	800	22
5	Mahua (*Mahuaindica*)	512	40.5
6	Khirni (*Manilkarahexandra*)	675	15.67
7	Manila tamarind (*Pithecellobiumdulce*)	25	138
8	Jamun (*Syzygiumcumini*)	80	18
9	Tamarind (*Tamarindusindica*)	-	44
10	Ber (*Zizyphus spp.*)	-	88

http://www.academicjournals.org/jhf/PDF/pdf2009/Sept/Rathore.pdf

A plant based diet protects against chronic oxidative stress related diseases. Dietary plant contains variable chemical families and amount of antioxidants. Berries, nuts, vegetables and products constituting these are high in their antioxidant values. This shows that plant-based foods introduce significantly more antioxidants into human diet than non-plant foods (Carlsen et al., 2010).

1.3 Antimicrobial activity

An antimicrobial substance is that, which kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoans. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substances used on nonliving objects. Fruit products are known to potent antimicrobial agents against bacteria and fungus. These fruits are rich source of flavonid and many polymethoxylated flavones which are very rare in other plants. These compounds, not only play an important and physiological role, but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries activities of the world (Mathur et al., 2011).

The most critical problem related with health and causes of mortality rates in society is infectious disease. Since last decade, there has been an increasing evidence of bacterial and fungal infections due to population explosion, pollution, changed environmental conditions and wastes from different sources, which may affect food with perfect nutrition value. These factors cause less immunogenicity in human beings and animals. This fact coupled with the resistance developed in microorganisms to allopathic agents and antibiotics with increased toxicity in human beings and animals during prolonged treatment with several antimicrobial drugs motivated scientists to find out new natural sources which can act as an antimicrobial biomolecules to treat opportunistic microbial infections (Movalia & Gajera, 2009).

According to world health organization (WHO), infectious diseases are the number one cause of the death worldwide. The emergence of antibiotic-resistant microorganisms had swiftly reversed the advances of previous fifty years of research on antibiotics (Menghani et al., 2011). Furthermore, some antibiotics have serious undesirable side effects which limit their applications (Shan et al., 2007). Hence, the ultimate goal of the leading drug companies and the academia is to hunt for novel therapeutic/antibacterial agents that are effective with minimal side effects (Sati et al., 2011). According to Ynalvez et al., (2012) a diverse range of bioactive compounds that offer potentials for the treatment of chronic and infectious diseases can be found most especially in traditional plants.

1.4 *Rasbhari* (*Physalis peruviana*)

Physalis peruviana belongs to family Solanaceaeans is also known as Cape gooseberry in English. The chief characteristics of this small, round and exotic berry is its pulp ranging from yellow to dark orange in color, and has form and structure similar to a tomato. The fruit may contain in between 150 and 300 seeds, with a diameter ranging between 12.5 and 25.0 mm and weighing from 4 to 9 g each .The fruit are partially or totally enclosed in a papery husk in the shape of a balloon, known as the calyx (Silvana et al., 2013).

Despite of the fact that this fruit crop has great potential in respect of production and market acceptability yet this crop has never been exploited for inclusion in the main stream of cultivated fruit crops and falls in the group of minor under exploited fruit crops (Ahmed et.al., 2011).

Many medicinal properties are attributed to *rasbhari* such as antispasmodic, diuretic, antiseptic, sedative, analgesic, helping to fortify the optic nerve, throat trouble relief, elimination of intestinal parasites and amoeba. There have also been reported antidiabetic properties, recommending the consumption of five fruits a day. So far,there are no studies that indicate possible adverse effects (Rodriguez & Rodrig, 2007).

Chang et al., (2008) conducted a study to examine the antioxidant activities of *rasbhari (Physalisperuviana).* The study concludes antioxidant activity and potent hepatoprotective effect against induced liver injury in rat. Using different models of antioxidant assay, namely ferric thiocyanate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and reducing power, *rasbhari* extract showed a dose-dependent increase in antioxidant activities, with total antioxidant activity (IC_{50}:0.81 µg/ml). The study concluded that *rasbhari* possesses antioxidant activity and potent hepatoprotective effect.

The fruit of *rasbhari* has been traditionally used as antispasmodic, diuretic, antiseptic, sedative, and analgesic. Park et al.,(2013) conducted a study on the qualitative content analysis of the *rasbhari* fruits to clarify the in vitro genotoxicity and in vivo acute and sub chronic toxicity of the fruit and concluded that lyophilized fruit juice of *rasbhari* is shown to induce cardiac toxicity only at high doses (more than 5000mg/kg)in male gender.

Yanartaş et.al., (2012) in the study of a manic episode induced by herbal preparation with *rasbhari* concluded that herbal preparation are widely used especially in treatment of cancer, losing weight, analgesia, or for other health problems in folk medicine. In psychiatric disorders they are used especially for insomnia, anxiety, depression, and sexual disorders.

1.5 *Jungle jalebi* (*Pithecellobiumdulce)*

Pithecellobiumdulce belongs to family Leguminoceae, is an evergreen tree widely distributed in the greater part of India and is also found in Southeast Asia . Plant locally is known by various names at different regions *viz*Hindi- *Vilayati babul, vilayatiimli, jangle jalebi*; Eng.- *Quamachil, Madras thorn, manila tamarind*; Beng.- *Dekhani babul*; Mar.- *Vilayati chinch*; Tel.- *Simachinta*; Tam.- *Kodukkaapuli*; Kan.- *Kottampuli, seemaehunase*; Mal.- *Korukkapuli*. The generic name is derived from the Greek word as '*Pithekos*' meaning an ape and lobos referring to a pod and the species name as '*dulce*' in Latin means sweet in allusion to the edible pulp of the pod. Because

of the resemblance of the fruits to the Indian sweet '*jalebi'*, the plant also given the name '*junglijalebi*' (*Indian MateriaMedica*, 1982).

The bark of the plant is reported to be used as astringent in dysentery, febrifuge and it is also useful in dermatitis and eye inflammation. The leaves have been reported to possess astringent, emollient, abortifiacient and antidiabeticproperties (Sugumaran et al., 2009).

The plant is well known for its edible fruits and they have been consumed for various ailments in a traditional manner. The fruits are linear, curved legumes (Pods) that range in length from 10 to 13 cm. The pod splits along both margins. The legumes may contain 5 to 12 seeds which are reddish brown to black in colour. The fruits turn from green to reddish brown when they ripen. The pod fragments can be eaten raw or made in to a drink for its nutritive as well as therapeutic values but still most of the chemical constituents of the pods are remained unexplored and underutilized(Pradeepa et al.,2014).

The ethnopharmacological importance of *jungle jalebi* is evidenced by its traditional use for gastric complications. The study conducted by Geetha, (2012) evaluated the gastroprotective activity and the mechanism of action of hydro alcoholic fruit extract of *jungle jalebi* (HAEPD) in rats by using chemical and stress induced ulcer models. It is concluded that HAEPD possess a potent antiulcer activity probably by acting as cytoprotective and antiacid secretory agent

Pradeepa et al.,(2013) was designed a study to evaluate the antidiabetic potential of *jungle jalebi* fruits in STZ induced experimental diabetes in rats. Phytochemical analysis of the fruit extract revealed the presence of alkaloids, flavonoids, glycosides, saponins and phytosterols. Oral administration of *jungle jalebi* fruit extract (300 mg/kg b.w. /day) to diabetic rats for 30 days significantly reduced the levels of blood glucose, glycosylated hemoglobin, urea and creatinine. The altered levels of serum aminotransferases and alkaline phosphatase were normalized upon treatment with the fruit extract. The observed decrease in the levels of plasma protein, plasma insulin, and hemoglobin in the diabetic rats were elevated to near normal by the extract treatment. The level of glycogen content was improved upon treatment with the extract. The results of the study indicated that the fruit extract is nontoxic and possess antidiabetic nature.

Manna, (2010) in a study investigated the beneficial role of the aqueous extract of the fruits of *jungle jalebi* against carbon tetrachloride (CCl_4)-induced hepatic injury. The aqueous extract of

jungle jalebi has been found to possess free radical (DPPH, hydroxyl and superoxide) scavenging activity in cell-free system. Treatment with aqueous extract of *jungle jalebi* both pre- and post-toxin exposure protected the organ from CCl_4-induced hepatic damage. The study concluded that aqueous extract of *jungle jalebi* protects murine liver against CCl4-induced oxidative impairments probably via its antioxidative property.

Magala, (2010) carried out a study to evaluate the acute and sub-acute toxicity profile of the hydroalcoholic fruit extract (HAEPD) of *jungle jalebi* . Albino rats were treated orally with 100, 200 and 500mg/kg bodyweight (BW) of HAEPD for 90 days to assess its sub-acute toxicity. HAEPD at single doses of 100, 500, 1000, 2000 and 4000mg/kg BW was also administered to rats to assess its acute toxicity. The rats were observed for physical discomfort, BW change and feeding habits. *Jungle jalebi* did not cause any abnormal changes in haematological or biochemical parameters. It is concluded that hydroalcoholic fruit extract of *jungle jalebi* can be used safely for experimental and clinicaltrials.

1.6 Significance of the study

In nature there are many underutilized fruits of promising nutritive value, which can nourish even the increasing human population. In India, various types of underutilized foods are available seasonally but are not utilized to the extent they should be in spite of their higher nutritive value. Looking into the prevalence of high level of micronutrient malnutrition among the vulnerable sections, utilization of underutilized foods can be explored to overcome nutritional disorders. They remain underutilized due to lack of awareness. Use of wild fruits as a food has decreased due to improvement and hybridization in commercially cultivated fruits. Any scientific evidence for the health benefits of such wild fruits in addition to their nutritional value would be a value addition to the plants producing such fruits.

Today, consumers are becoming increasingly conscious of the health and nutritional aspects of their food basket. The tendency is to avoid chemicals and synthetic foods and preference for nutrition through natural resources. The underutilized fruits are good in their therapeutic and nutritive value and can satisfy the demands of the health-conscious consumers. Nowadays, considerable interest is focused on the development and evaluation of natural antioxidants and antimicrobial abilities from plant materials which are rich in polyphenolic compounds. It is well known that plant derived polyphenols have remarkable antioxidant and free radical scavenging

activity resulting in multiple beneficial nutritional physiological effects in the humans. The antimicrobial abilities of underutilized fruits are shown to be of particularly interesting field for applications within the food industries. Hence, there is a need to concentrate on research efforts in diversification and popularization of such underutilized fruit crops.

In the present study an effort will be made to unfold the beneficial abilities like, antioxidant and antimicrobial properties of lesser known fruits - *rasbhari* and *jungle jalebi*.

Keeping this in mind, present study has been undertaken with the following objectives-

1.7 Objectives

- To determine sensory characteristics of the selected fruits - *Rasbhari* (Physalisperuviana) and *Jungle Jalebi* (*Pithecellobium dulce*).
- To estimate nutrient content of the two selected fruits.
- To analyse antioxidant (nutrient and non nutrient) content of the selected fruits.
- To determine the antioxidant and antimicrobial activities of the selected fruits.

2. Methodology

For the present study two underutilized fruits, namely *rasbhari* (*Physalis peruviana*) and *jungle jalebi* (*Pithecellobium dulce*) will be selected for their nutrient and antioxidant (nutrient and non nutrient) analysis, as well as, for determining their antioxidant and antimicrobial activities. The study will be carried out in the following phases -

2.1 Phase-I

Procurement and pre preparation of fruits will be carried out in this phase.

2.1.1 *Procurement of the selected fruits*

The fruits, *rasbhari* and *jungle jalebi,* will be procured from the Horticulture Centres of Jaipur and Ajmer, Rajasthan. After procurement the fruits will cleaned by removing the rotten and damaged pieces of fruits and thereafter will be washed 2-3-times with running tap water, dried and stored under suitable temperature till use.

2.2 Phase-II

Phase II will include processing of fruits for further analyzing difference between fresh and processed fruits related to nutrient and antioxidant retention. The processing techniques used will be 'blanching' and 'drying'. Blanching is a unit operation prior to freezing, canning or drying in which fruits are heated for the purpose of inactivating enzymes, modifying texture, preserving colour, flavour, nutritional value and removing trapped air. Blanching of fruits, using hot air oven helps in retaining ascorbic acid and carotene (Corcuera, 2004).

2.2.1 *Processing of rasbhari and jungle jalebi*

The fruits will be sliced, thereafter blanched in a water bath at 90º C for 10 minutes and cooled in cold water (10-12ºC) for 60 seconds (Yan et al., 2010).The surface water of blanched fruits will be removed by clean cloth, then will be homogenized by using mechanical blender. The blanched fruits will then be dried and ground to fine powder (Rangana, 2010) for further estimations.

2.2.2 *Drying of fresh rasbhari and jungle jalebi*

The edible fruit part of fresh fruits will be homogenized by using mechanical blender. The fruits will then be dried in a hot air oven and ground to fine powder (Rangana, 2010) for nutrient estimations.

2.3 Phase-III

Proximate nutrient analysis will be carried out in this phase.

2.3.1 *Nutrient analysis*

Nutrients like moisture, protein, ash, crude fiber and fat will be estimated using standard techniques. Carbohydrate will be determine by subtracting from 100 the sum of the values(per 100 g) for moisture, protein, ash, crude fiber and fat .These nutrients will be analyzed for both, fresh and processed fruit samples. Five samples of each fruit will be analyzed for all these nutrients. Each sample will be estimated in triplicate.

Table 1

Methods to be used for Nutrients analysis of *rasbhari* and *jungle jalebi* (fresh and processed)

S. No.	Name of nutrient	Methods	References
1	Moisture (g/100g)	Oven drying method	AOAC, 2005
2	Protein (g/100g)	Micro-kjeldhal method	AOAC, 2005

3	Ash content(g/100g)	Muffle Furnace method	AOAC, 2005
4	Crude fiber (g/100g)	Acid-alkali treament	AOAC, 2005
5	Fat (g/100)	Ether Extractive	AOAC, 2005
6	Carbohydrate (g/100g)	Calculation based	AOAC, 2005

2.4 Phase-IV

Analysis of antioxidant (nutrient and non nutrient) will be carried out in this phase.

2.4.1 ***Analysis of nutrient antioxidant of rasbhari and jungle jalebi (fresh and processed)***

Analysis of antioxidants (β-carotene, Vitamin C and Vitamin E) of both fresh and processed *rasbhari* and *jungle jalebi* will be carried out by using standard techniques.

Table 2

Methods to be used for analysis of nutrient antioxidant of *rasbhari* and *jungle jalebi* (fresh and processed)

Name of nutrient antioxidant	**Methods**	**References**
1.β-carotene (µg/100g)	HPLC	NIN,2003
2.Vitamine C(mg/100g)	Titration method	AOAC, 2005
3.Vitamin E(mg/100g)	HPLC	NIN,2003

2.4.2 ***Analysis of non nutrient antioxidant content of rasbhari and jungle jalebi (fresh and processed)***

Analysis of total polyphenol and total flavonoid content of *rasbhari* and *jungle jalebi (*fresh and processed*)* will be carried out by using standard methods.

Table 3

Methods to be used for Analysis of antioxidants (non nutrient) of *rasbhari* and *jungle jalebi* (fresh and processed)

Name of non nutrient antioxidant	**Method**	**References**
Total Polyphenol	Folin-Ciocalteu method	Singleton et.al ,1999
Total Flavonoid	Aluminum chloride spectrophotometric assay	Ordonez et al ,2006

Preparation of extract for non nutrient antioxidant analysis

Known quantity of pulp powder will be extracted with petroleum ether (60-80˚C) to remove wax and then extracted with 80% ethanol in a soxhlet apparatus. The solvent will be evaporated to dryness in a rotary evaporator at reduced pressure below 40˚C (Rangana,2010). The extract then will be used for further experiments.

2.4.3 *Assesssment of toxicity of rasbhari and jungle jalebi*

Toxicity of *rasbhari* and *jungle jalebi* will be assessed by comparing the values of analyzed nutrients (per 100gm) with the recommended dietary allowances of nutrients (per 100gm) given by ICMR,1989.

2.5 Phase-V

Antioxidant activity of *rasbhari* and *jungle jalebi* (fresh and processed) will be determine in this phase.

2.5.1 *Determination of antioxidant activity of rasbhari and jungle jalebi (fresh and processed)*

Determination of antioxidant activity of *rasbhari* and *jungle jalebi* (fresh and processed) will be carried out by using standard techniques. The extract for this estimation will be prepared in the same way as will be prepared for estimating non nutrient antioxidants.

Table 4

Methods to be used in Determination of antioxidant activity of *rasbhari* and *jungle jalebi* (fresh and processed)

Method	**References**
1,1-diphenyl-2-picrylhydrazyl (DPPH) assay	Sharma and Bhat,2009
Ferric Reducing Antioxidant Power (FRAP) assay	Benzie and Strain,1996

2.6 Phase-VI

Antimicrobial activity of *rasbhari* and *jungle jalebi* (fresh and processed) will be carried out in this phase.

2.6.1 *Determination of antimicrobial activity of rasbhari and jungle jalebi (fresh and processed)*

Two Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus epidermidis*) and two Gram negative bacteria (*Escherichia coli*, *Salmonella typhi)* will be used to determine the antimicrobial activity of fresh and processed test samples. The extract for this estimation will be prepared in the same way as will be prepared for estimating non nutrient antioxidants.

Table 5

Method to be used in determination of antimicrobial activity of rasbhari and jungle jalebi (fresh and processed).

Method	**Reference**
Agar well diffusion method	Perez *et al.*,1990
Minimum inhibitory concentration (MIC)	Patel, 2009

2.7 Phase-VII

Sensory characteristics of fresh *rasbhari* and *jungle jalebi* will be determine in this phase.

Sensory characteristics of fresh rasbhari and *jungle jalebi* will be determine by using sensory evaluation for their taste and acceptability.

2.8 Phase-VII

Statistical Analysis of data will be carried out in this phase.

The mean and standard deviation of all the estimated values related to nutrient content, antioxidants (nutrient and non nutrient), antioxidant and antimicrobial activities will be calculated. The least significant difference tests (t-test, ANOVA) will be used to determine difference between means of fresh and processed *rasbhari* and *jungle jalebi.*

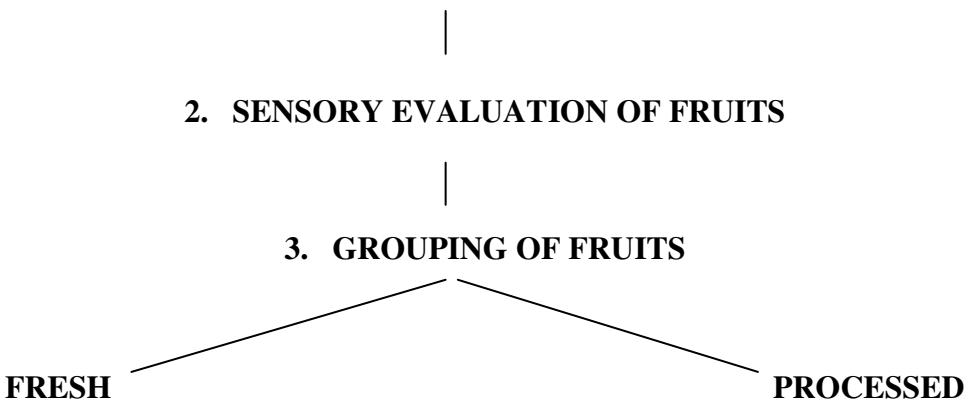
2.9 Phase-VIII

Report writing

STUDY DESIGN

1. PROCUREMENT OF FRUITS

***Rasbhari* and *Jungle jalebi* from the Horticulture Center, Jaipur and Ajmer, Rajasthan**



Rasbhari* and *jungle jalebi

- Hot Air Oven drying

Rasbhari* and *jungle jalebi

- Blanching
- Hot Air Oven drying

4. NUTRIENT ANALYSIS

Moisture,
Protein
Ash
Crude fiber
Fat
Carbohydrate

5. ANTIOXIDANT (Nutrient) ANALYSIS

β-carotene
Vitamine C
Vitamin E

6. ANTIOXIDANT (Non-nutrient) ANALYSIS

Total Polyphenol

Total Flavonoid

7. ANTIOXIDANT ACTIVITIES

8. ANTIMICROBIAL ACTIVITIES

Gram positive bacteria
Bacillus subtilis
Staphylococcus epidernidis
Gram negative bacteria

Escherichia coli
Salmonella typhi

9. STATISTICAL ANALYSIS

10. THESIS WRITING

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Analysis of Nutritional Quality and Evaluation of Antioxidant and Antimicrobial Activities of *Rasbhari* (*Physalis peruviana*) and *Jungle jalebi* (*Pithecellobiumdulce)*

Submitted

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Doctor of Philosophy

in the Faculty of Science

Home science

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BY:

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